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Marasmioid and gymnopoid fungi of the Republic of Korea. 3. Two new taxa of *Marasmius* sect. *Sicci* with caulocystidia and/or setae

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Abstract — Two new species of *Marasmius* sect. *Sicci* are described. *Marasmius orientalis,* which is characterized by having a pileipellis with well-developed setoid broom cells and setae, caulosetae present, belongs to series *Spinulosi*. *Marasmius strobiluriformis* has dimorphic cheilocystidia and cylindrical to subfusoid caulocystidia and belongs to ser. *Atrorubentes*. The macro- and microscopic descriptions with discussion on similar taxa are given, and their taxonomic position is supported through DNA analyses.

Key words — euagarics, *Marasmiaceae*, taxonomy, nomenclature, LSU, ITS

Introduction

This paper is a part of a series dealing with the marasmioid and gymnopoid fungi of the Republic of Korea studied within a joint project (Antonín et al. 2009, 2010). During joint field excursions, many collections of those fungi were collected, including several new taxa. Two of them, belonging to section *Sicci* with well-developed setae and/or caulocystidia (series *Spinulosi* and ser. *Atrorubentes*), are described here.

Material and methods

Macroscopic descriptions of collected specimens are based on fresh basidiocarps and photos made by the second author. Microscopic features are described from dried

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material mounted in H_2O, KOH, Melzer's reagent and Congo Red using an Olympus BX-50 light microscope with a magnification of 1000×. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (mean of E-values) are used. For lamellae, L means number of entire lamellae and l means number of lamellulae between each pair of entire lamellae. Authors of fungal names are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/ authorsearchpage.do). Herbarium specimens are preserved in the herbarium of the Moravian Museum, Brno, Czech Republic (BRNM).

Genomic DNA was extracted using a small piece (3–4 mm^3) of the dried basidiocarp. The DNA extraction was made according to Lee & Taylor (1990). PCR amplification of nLSU rDNA region was made according to the modified method by Moncalvo et al. (2000). PCR primer LR0R and LR7 were used for the selective amplification of nLSU rDNA region. PCR amplification of ITS rDNA region was performed according to the modified method described by Douanla-Meli & Langer (2008). Forward primer ITS1-F and reverse primer ITS4-B were used for the selective amplification of ITS rDNA region. Purified DNAs were directly sequenced on an ABI Prism TM 377 DNA Automatic Sequencer (Applied Biosystems, Foster City, CA, USA) using BigDye™ cycle sequencing kit, version 3.1 (Applied Biosystems). Primers identical with amplification for ITS rDNA whereas LR0R, LR7 and additional internal primer LR3 for LSU rDNA were used.

Sequences were edited with the DNASTAR software (Lasergene, Madison, Wis.). An alignment of the sequences was performed using the CLUSTAL X (Thompson et al. 1997). Phylogenetic trees were obtained from the data using Bayesian modelling (Geyer 1991; MRBAYES, version 3.0b4, Ronquist & Huelsenbeck 2003). For a given data set, the general time reversible (GTR) model was employed with gamma-distributed substitution rates. Markov chains were run for 2,000.000 generations, saving a tree every 100th generation. Among these, the first 1000 trees were discarded as burn-in phase of each analysis. MRBAYES was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities (PPs) of the groups. The type species of the genus *Marasmius*, *M. rotula* (Scop. : Fr.) Fr. (sect. *Marasmius*), was selected as the outgroup.

Taxonomy

Marasmius orientalis Antonín, R. Ryoo & H.D. Shin, **sp. nov.** Fig. 1

MycoBank MB 515673

NCBI accession numbers: BRNM 714913 [GU266262 (ITS), GU266269 (LSU)]

Pileo usque 29 mm lato, conico, campanulato, obtuso, striato-sulcato, marginem crenulato, albido vel cremeo, centro brunneo. Lamellis distantibus, cremeis. Stipite ca. 60 × 1.5 mm, cylindraceo, subtiliter pubescente, ad apicem albido vel cremeo, ad basin (aurantiaco-)brunneo. Basidiosporis 9.0–10.5 × 4.5–6.0 µm, ellipsoideis, ellipsoideo-fusiformibus. Cheilocystidiis 16–28 × 5.0–9.0 µm, clavatis, (sub)cylindraceis, subvesiculosis, saepe irregularibus, tenuitunicatis. Pleurocystidiis 40–45 × 7.0–10 µm, fusiformibus, cylindraceis, rostratis, tenuitunicatis. Pileipellis hymeniformis, e cellulis (15–)24–40 × (8.0–)10–12 µm, clavatis, pyriformibus vel subcylindraceis, laevibus; pileosetis et cellulis similibus cellularum hymenidermatis Marasmii sicci, crassitunicatis praesentis. Caulosetis 35–70 × 6.0–9.0(–12) µm, fusiformibus, lageniformibus, crassitunicatis luteo-brunneis. Hyphis fibulatis, in stipite et trama dextrinoideis.

Holotype: *Republic of Korea, Seoul, park nationalis Bukhansan, apud rivi Jeongreung, 17. VII. 2008, leg. R. Ryoo KG 255 (**holotypus** in herbario BRNM 714913 asservatur).*

Etymology: from the Latin *oriens* = east

Basidiocarps single. Pileus up to c. 25 mm broad, broadly conical or high-campanulate with obtuse centre and inflexed crenulate margin, striate-sulcate, slightly tomentose, off-white to cream with brown centre. Lamellae distant, L = c. 18, l = 1, emarginate and with small tooth, cream with concolorous edge. Stipe c. 60 × 1.5 mm, cylindrical, minutely pubescent, non-insititious, white or cream above, (orange-)brown towards base. (Description according to one photo and dry basidiocarp).

Basidiospores 9.0–10.5 × 4.5–6.0 µm, average = 9.8 × 5.2 µm, E = 1.7–2.1, Q = 1.9, ellipsoid, ellipsoid-fusoid, ellipsoid-lacrimoid, smooth, hyaline, non-dextrinoid, both thin-walled and slightly thick-walled. Basidia not found. Basidioles up to 45 × 4.0–8.0 µm, cylindrical, clavate. Cheilocystidia 16–28 × 5.0–9.0 µm, clavate, (sub)vesiculose, subcylindrical, often irregular, smooth, sometimes with apical projections, thin-walled. Pleurocystidia 40–45 × 7.0–10 µm, fusoid or subcylindrical, rostrate, refractive, thin-walled, sometimes originating in subhymenium and not or only slightly projecting beyond hymenium. Trama hyphae ± cylindrical, thin- to slightly thick-walled, smooth or minutely incrusted, hyaline, dextrinoid, up to 12 µm wide. Pileipellis a hymeniderm composed of (15–)24–40 × (8.0–)10–12 µm, clavate, pyriform, subcylindrical, smooth, thin-walled with often slightly thick-walled central part, hyaline to yellow-brown in KOH; mixed with setoid broom cells and setae, cylindrical to clavate, or fusoid, subulate or sublageniform, thick-walled, weakly dextrinoid, yellow-brown in KOH, with up to 35 × 3.0 µm, obtuse to subacute projections. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, smooth, dextrinoid, up to 5.0 µm wide hyphae with yellow-brown walls in KOH. Caulosetae 35–70 × 6.0–9.0(–12) µm, fusoid, lageniform, subulate, often branched, thick-walled (walls up to 1.0 µm), obtuse to acute, weakly dextrinoid, with yellow-brown walls in KOH. Clamp connections present in all tissues.

Habitat — On detritus of broadleaf trees.

Remarks — *Marasmius orientalis* is characterized by having a broadly conical, striate-sulcate, off-white to cream coloured pileus with a brown centre, rather small basidiospores, mostly smooth cheilocystidia, well-developed fusoid or subcylindrical pleurocystidia, pileipellis of smooth cells with well-developed setoid broom cells and setae and present caulosetae. Having well-developed setae, it belongs to ser. *Spinulosi* (Clémençon) Desjardin.

Marasmius torquescens Quél., widespread in Europe, is a very similar species. It differs only by a never sulcate and high-campanulate pileus, slightly different cheilocystidia, and smaller pleurocystidia (Antonín & Noordeloos

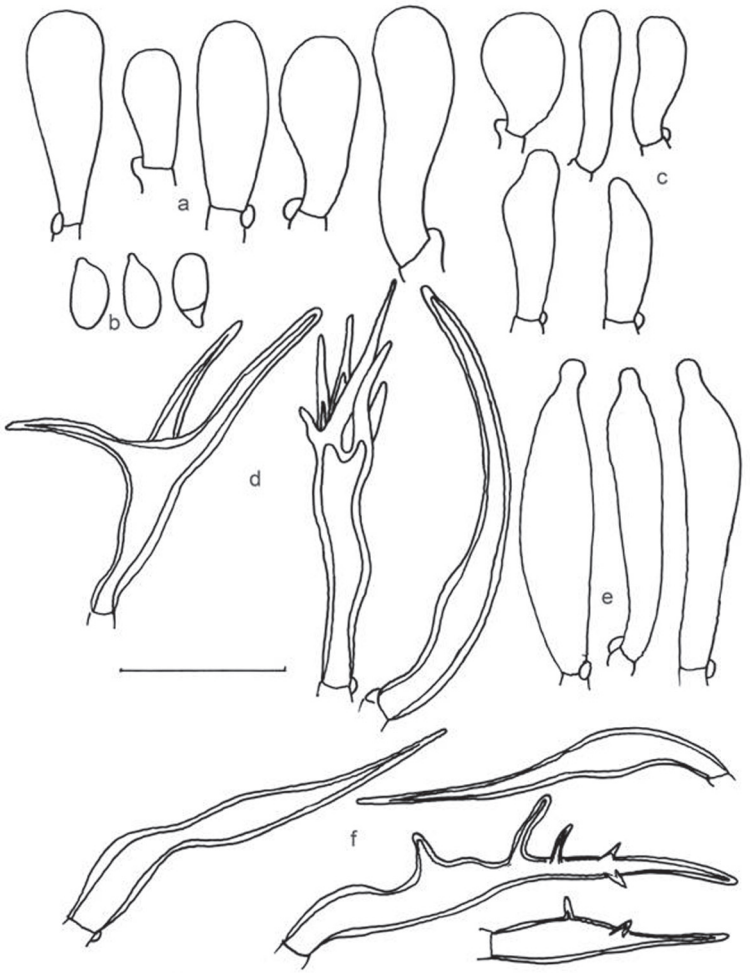

Fig. 1. *Marasmius orientalis*.
a) pileipellis cells, b) basidiospores, c) cheilocystidia,
d) pileus setoid broom cells and pileosetae, e) pleurocystidia, f) caulosetae.
Scale bar = 20 µm.

2010). *Marasmius torquescens* and *M. orientalis* significantly differ in DNA sequences. Macroscopically similar *M. delectans* Morgan and *M. cohaerens* var. *lachnophyllus* (Berk.) Gilliam differ especially by having hymenial setae and ± different pileipellis structure (Gilliam 1976). *Marasmius mokfaensis* Wannathes et al. has distinctly larger basidiocarps, larger basidiospores (27–33 x 5–6 µm), and absent pleurocystidia and setae (Wannathes et al. 2009).

Marasmius strobiluriformis Antonín, R. Ryoo & H.D. Shin, **sp. nov.** Fig. 2

MycoBank MB 515674

NCBI accession numbers: BRNM 714914 [GU266263 (ITS), GU266270 (LSU)]; BRNM 714915 [GU266264 (ITS), GU266271 (LSU)]

Pileo usque ca. 12 mm lato, convexo-hemisphaerico vel plano-convexo, obtuso, albo. Lamellis albis. Stipite ca. 40 × 1 mm, cylindraceo, subtiliter pubescente, apicem albido, ad basin aurantiaco-brunneo. Basidiosporis (9.0–)10–14 × 4.0–5.5 µm, fusiformibus, raro ellipsoideo-fusiformibus. Cheilocystidiis (1) e cellulis similibus cellulis typo Marasmii sicci, 13–23 × 6.0–13 µm, clavatis, pyriformibus, subvesiculosis, subcylindraceis, tenuitunicatis, (2) 14–32 × 4.5–10(–14) µm, fusiformibus, cylindraceis, clavatis, vesiculosis, glabris vel cum projectionibus apicalibus, tenuitunicatis. Pileipellis hymeniformis, e cellulis similibus cellulis hymenodermatis Marasmii sicci, 10–18 × 6.0–9.0 µm, clavatis, pyriformibus, subfusiformibus vel subcylindraceis, tenuitunicatis. Caulocystidiis 25–55 × 5.0–8.0 µm, cylindraceis vel subfusiformibus, leviter irregularibus, leviter crassitunicatis. Hyphis fibulatis, in stipite et trama dextrinoideis.

Holotype: *Republic of Korea, Chuncheon, Dongsan-myon, Bongmyong-ri, 20. VIII. 2007 leg. R. Ryoo KG 142 (**holotypus** in herbario BRNM 714914 asservatur).*

Etymology: basidiocarps similar to the genus *Strobilurus*

Basidiocarps single. Pileus up to c. 12 mm broad, convex-hemispherical, then plano-convex with obtuse centre and involute, then inflexed margin, smooth, slightly tomentose, white. Lamellae moderately close, L = 15–17, l = 3, emarginate, white with concolorous, finely pubescent edge. Stipe c. 40 × 1 mm, cylindrical, minutely pubescent, non-insititious, white above, orange-brown towards base.

Basidiospores (9.5–)10–14(–15) × 4.0–5.5 µm, average = 12 × 4.6 µm, E = 2.0–3.3, Q = 2.3–3.0, fusoid, less frequently ellipsoid-fusoid, smooth, hyaline, non-dextrinoid, thin-walled. Basidia not found. Basidioles up to 35 × 3.0–9.0 µm, cylindrical, subfusoid, clavate. Cheilocystidia of two types, (1) in the form of broom cells of the Siccus-type, 13–23 × 6.0–13 µm, clavate, pyriform, subvesiculose, subcylindrical, thin-walled; projections up to 25, digitate, nodulose, obtuse to (sub)acute, thin to slightly thick-walled, up to 7.0 × 1.0 µm, and (2) fusoid, cylindrical, clavate, vesiculose, 14–32 × 4.5–10(–14) µm, smooth or with ± large apical projections, not refractive, thin-walled. Pleurocystidia absent. Trama hyphae ± cylindrical, thin- to slightly thick-walled, hyaline, dextrinoid, up to 20 µm wide. Pileipellis a hymeniderm composed of broom cells of the Siccus-type, 10–18 × 6.0–9.0 µm, clavate, pyriform, subcylindrical, subfusoid, thin-walled; projections up to 25, digitate, nodulose, obtuse to subacute, thin- to distinctly thick-walled, up to 8.0 × 1.0 µm, hyaline. Pileocystidia absent. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, smooth, dextrinoid, up to 5.0(–6.0) µm wide hyphae. Caulocystidia single or in groups, 25–55 × 5.0–8.0 µm, erect, suberect or appressed, cylindrical to subfusoid, slightly irregular, slightly

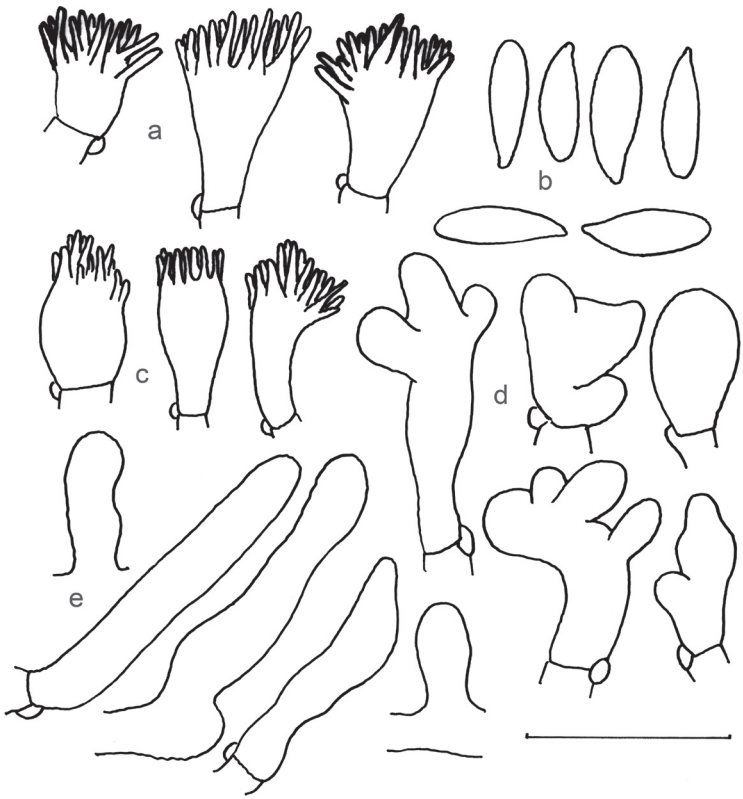

Fig. 2. *Marasmius strobiluriformis*.
a) pileipellis cells, b) basidiospores, c) cheilocystidia of the type 1,
d) cheilocystidia of the type 2, e) caulocystidia.
Scale bar = 20 µm.

thick-walled, dextrinoid, with hyaline to pale yellowish walls in KOH. Clamp connections present in all tissues.

Habitat — On detritus under *Pinus* and *Quercus* sp.

Additional collection — Republic of Korea, Chuncheon, Dongsan-myeon, Bongmyeong-ri, 20 Aug. 2008 leg. R. Ryoo KG 250 (BRNM 714915).

Remarks — *Marasmius strobiluriformis* is characterized by having a small, convex-hemispherical, then plano-convex, white pilei, rather small basidiospores, dimorphic cheilocystidia (broom cells and rostrate or (often) at apex branched ones), and cylindrical to subfusoid caulocystidia. Due to

the presence of caulocystidia, it belongs to ser. *Atrorubentes* Desjardin & E. Horak.

Among similar species, *Marasmius pseudoniveus* Singer has a larger, 20–40 mm broad, sulcate pilei and smaller (7.5–9 × 3–4 μm) basidiospores (Pegler 1997), *M. halimunensis* Desjardin et al. has a similarly coloured but striate pileus, smaller basidiospores (11–12 × 4 μm), more variable cheilocystidia in shape, and projections and pileipellis consisting of smooth and broom cells (Desjardin et al. 2000), and *M. subarborescens* Singer has a larger, 8–35 mm broad pileus, very close, narrow lamellae, ochraceous-brown stipe at base, smaller basidiospores (6.0–8.0(–9.0) × 2.7–3.2 μm) and dimorphic cheilocystidia in the form of smooth and broom cells (Antonín 2007).

Phylogenetic analysis

Based on ITS and LSU rDNA sequences obtained in this study and from GenBank, the phylogenetic relationships of *Marasmius orientalis* and *M. strobiluriformis* were inferred from Bayesian (MCMC) analyses. ITS and LSU sequences were aligned and the ends trimmed to create a dataset of 656 and 981 base pairs, respectively.

The resulting phylogenetic trees of ITS and LSU rDNA data are shown in Figs. 3 and 4. The independent taxonomic status of two new species was

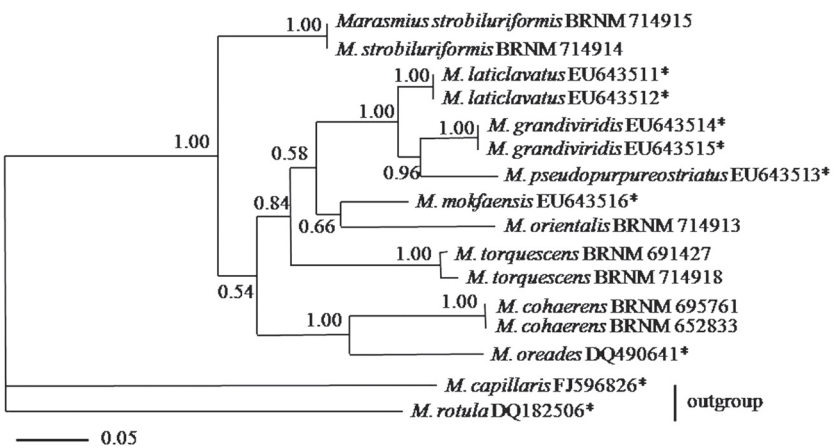

Fig. 3. Phylogenetic tree for *Marasmius orientalis* and *M. strobiluriformis* on the ITS (ITS1, 5.8S rDNA, and ITS2) rDNA region, showing mean branch lengths of a 50 % majority rule consensus tree, obtained from an MCMC analysis of two million generations. An asterisk (*) denotes a sequence from GenBank. Bar = 0.05

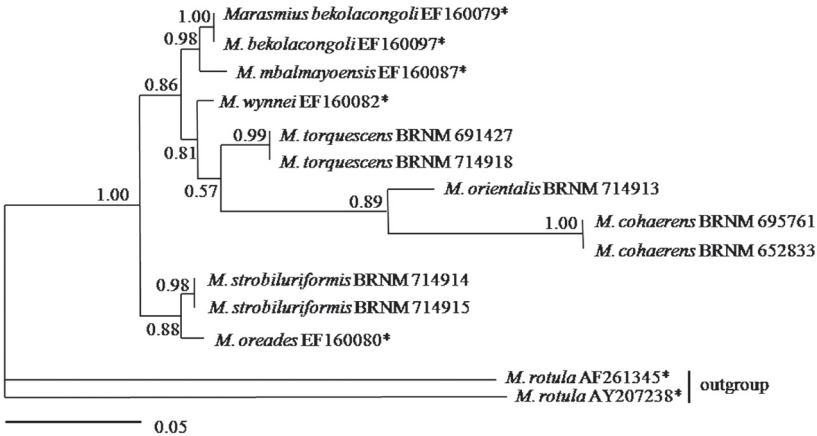

Fig. 4. Phylogenetic tree for *Marasmius orientalis* and *M. strobiluriformis* on the nLSU rDNA (nuclear large subunit ribosomal DNA) sequences, showing mean branch lengths of a 50 % majority rule consensus tree, obtained from an MCMC analysis of two million generations. An asterisk (*) denotes a sequence from GenBank. Bar = 0.05

supported by high posterior probability values. In this study, not only each new species but also *Marasmius torquescens* BRNM 691427 [GU266258 (ITS), GU266265 (LSU)], BRNM 714918 [GU266259 (ITS), GU266266 (LSU)] and *M. cohaerens* BRNM 695761 [GU266260 (ITS), GU266267 (LSU)], BRNM 652833 [GU266261 (ITS), GU266268 (LSU)] were sequenced for the first time. The newly obtained sequences will be analyzed in further study.

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